

WE CLAIM:

1. A method of enhancing cardiac function in a mammal, comprising delivering a vector to the heart of said mammal, the vector comprising a gene encoding a beta-adrenergic signaling protein (beta-ASP) operably linked to a promoter.

5 2. A method of enhancing cardiac function according to claim 1, wherein the vector is introduced into a blood vessel supplying blood to the myocardium of the heart.

3. A method of enhancing cardiac function according to claim 1, wherein the vector is delivered to cardiac myocytes.

10 4. A method of enhancing cardiac function according to claim 2, wherein said blood vessel supplying blood to the myocardium of the heart is a coronary artery, a saphenous vein graft or an internal mammary artery graft.

15 5. A method of enhancing cardiac function according to claim 4, wherein the vector is introduced into both left and right coronary arteries.

6. A method of enhancing cardiac function according to claim 1, wherein said mammal is a human.

20 7. A method of enhancing cardiac function according to claim 1, wherein the vector comprises a gene encoding a beta-ASP selected from the group consisting of a beta-adrenergic receptor (beta-AR), a G-protein receptor kinase inhibitor (GRK inhibitor) and an adenylylcyclase (AC).

8. A method of enhancing cardiac function according to claim 1, wherein the vector comprises genes encoding two different beta-adrenergic signaling proteins operably linked to a promoter.

25 9. A method of enhancing cardiac function according to claim 1, further comprising introducing a second vector comprising a gene encoding a second beta-ASP operably linked to a promoter, wherein said second beta-ASP is different from said first beta-ASP.

10. A method of enhancing cardiac function according to claim 7, wherein the beta-ASP is selected from the group consisting of a beta₁-adrenergic receptor (beta₁-AR) and a beta₂-adrenergic receptor (beta₂-AR).

11. A method of enhancing cardiac function according to claim 10, wherein the beta-ASP is a beta₁-adrenergic receptor (beta₁-AR).

12. A method of enhancing cardiac function according to claim 7, wherein the gene encoding a beta-ASP is a gene encoding a GRK inhibitor.

5 13. A method of enhancing cardiac function according to claim 7, wherein the beta-ASP is an adenylylcyclase (AC).

14. A method of enhancing cardiac function according to claim 13, wherein the beta-ASP is AC isoform VI.

10 15. A method of enhancing cardiac function according to claim 14, wherein the AC isoform VI comprises the amino acid sequence of SEQ ID NO. 13.

16. A method of enhancing cardiac function according to claim 15, wherein the gene encoding the AC isoform VI comprises the nucleotide sequence of SEQ ID NO. 12.

17. A method of enhancing cardiac function according to claim 13, wherein the beta-ASP is human AC isoform VI.

18. A method of enhancing cardiac function according to claim 13, wherein the beta-ASP is the human AC isoform VI of SEQ ID NO. 11.

15 19. A method of enhancing cardiac function according to claim 7, wherein the gene encoding a beta-ASP is operably linked to a heterologous promoter selected from the group consisting of a heterologous constitutive promoter and a heterologous inducible promoter.

20 20. A method of enhancing cardiac function according to claim 19, wherein the promoter is selected from the group consisting of a ventricular myosin light chain 2 promoter and a ventricular myosin heavy chain promoter.

25 21. A method of enhancing cardiac function according to claim 19, wherein the gene encoding a beta-ASP is a gene encoding AC isoform VI operably linked to a heterologous promoter.

22. A method of enhancing cardiac function according to claim 19, wherein the gene encoding a beta-ASP is the gene of SEQ ID NO. 10 encoding human AC isoform VI operably linked to a heterologous promoter.

23. A method of enhancing cardiac function according to claim 19, wherein the gene encoding a beta-ASP is a modified AC isoform VI gene operably linked to a heterologous promoter.

5 24. A method of enhancing cardiac function according to claim 23, wherein the modified AC isoform VI gene encodes a polypeptide comprising the amino acid sequence of SEQ ID NO. 13.

25. A method of enhancing cardiac function according to claim 1, wherein the gene encoding a beta-ASP is a variant of a wild-type beta-ASP gene, the variant comprising a deletion in one or more untranslated regions of said beta-ASP gene.

10 26. A method of enhancing cardiac function according to claim 25, wherein said deletion removes at least about 100 bp of the 3'-untranslated region.

27. A method of enhancing cardiac function according to claim 25, wherein the gene encoding a beta-ASP is a variant AC gene having a deletion in the 3'-untranslated region.

15 28. A method of enhancing cardiac function according to claim 25, wherein the gene encoding a beta-ASP is a truncated AC_{VI} gene having a deletion removing the 3'-untranslated region.

20 29. A method of enhancing cardiac function according to claim 1, wherein the vector is selected from the group consisting of a viral vector and a lipid-based vector.

30. A method of enhancing cardiac function according to claim 1, wherein the vector is a viral particle.

25 31. A method of enhancing cardiac function according to claim 30, wherein the viral particle is selected from the group consisting of an adenovirus (Ad) and an adeno-associated virus (AAV).

32. A method of enhancing cardiac function according to claim 31, wherein the viral particle is an adenovirus comprising a polynucleotide having a promoter operably linked to a gene encoding a beta-ASP, and said adenovirus vector is replication-defective in humans.

33. A method of enhancing cardiac function according to claim 32, wherein the beta-ASP is an adenylylcyclase (AC) isoform VI.

34. A method of enhancing cardiac function according to claim 32, wherein the beta-ASP is a modified AC isoform VI of SEQ ID NO. 13.

35. A method of enhancing cardiac function according to claim 32, wherein the beta-ASP is the human AC isoform VI of SEQ ID NO. 11.

5 36. A recombinant replication-defective viral particle comprising a gene encoding a beta-ASP operably linked to a promoter.

37. A recombinant replication-defective viral particle according to claim 36, wherein said promoter is a heterologous promoter.

10 38. A recombinant replication-defective viral particle according to claim 36, wherein said beta-ASP is selected from the group consisting of a beta-AR, a GRK inhibitor and an adenylylcyclase.

39. A recombinant replication-defective viral particle according to claim 36, wherein the vector comprises genes encoding two different beta-ASPs.

40. A recombinant replication-defective viral particle according to claim 36, wherein the beta-ASP is a beta₁-adrenergic receptor (beta₁-AR).

15 41. A recombinant replication-defective viral particle according to claim 36, wherein the beta-ASP is selected from the group consisting of AC isoform II, AC isoform V and AC isoform VI.

20 42. A recombinant replication-defective viral particle according to claim 36, wherein the beta-ASP is AC isoform VI.

43. A recombinant replication-defective viral particle according to claim 36, wherein the beta-ASP is a chimeric AC.

25 44. A recombinant replication-defective viral particle according to claim 36, wherein the beta-ASP is the AC isoform VI of SEQ ID NO. 13.

45. A recombinant replication-defective viral particle according to claim 36, wherein the beta-ASP is human AC isoform VI.

46. A recombinant replication-defective viral particle according to claim 36, wherein the beta-ASP is human AC isoform VI of SEQ ID NO. 11.

47. A recombinant replication-defective viral particle according to claim 36, wherein the gene encoding a beta-ASP is a variant of a wild-type beta-ASP gene, the variant comprising a deletion in one or more untranslated regions of said beta-ASP gene.

5 48. A recombinant replication-defective viral particle according to claim 47, wherein said deletion removes at least about 100 bp of the 3'-untranslated region.

49. A recombinant replication-defective viral particle according to claim 47, wherein the gene encoding a beta-ASP is a variant AC gene having a deletion in the 3'-untranslated region.

10 50. A recombinant replication-defective viral particle according to claim 47, wherein the gene encoding a beta-ASP is a truncated AC_{V1} gene having a deletion removing the 3'-untranslated region.

51. A recombinant replication-defective viral particle according to claim 36, wherein said recombinant replication-defective viral particle is an adenovirus that is replication-defective in humans.

52. A mammalian cell transfected with a recombinant replication-defective viral particle according to claim 36.

53. A filtered adenovirus particle preparation comprising:
15 (i) a recombinant replication-defective adenovirus particle according to claim 36, and
20 (ii) a carrier.

54. A filtered injectable adenovirus particle preparation according to claim 53, wherein said adenovirus vector has been filtered through a 0.1-0.5 micron filter.

55. A method of generating a recombinant replication-defective viral particle according to claim 36, comprising the following steps in the order listed:

25 (i) introducing first and second plasmids into a replication-permissive mammalian cell expressing one or more adenovirus genes conferring replication competence, wherein said first plasmid comprises a gene encoding a beta-ASP operably linked to a promoter and further comprises a replication-defective human adenovirus genome, and wherein said second plasmid comprises a replication-proficient human adenovirus genome and further comprises an additional polynucleotide sequence making the second plasmid too large to be
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encapsidated in an adenovirus particle, whereby rescue recombination takes place between the first plasmid and the second plasmid to generate a recombinant adenoviral genome comprising the gene encoding a beta-ASP but lacking one or more adenoviral replication genes, said recombinant genome being sufficiently small to be encapsidated in an adenovirus particle;

5 (ii) identifying successful recombinant viral vectors in cell culture; and
(iii) propagating a resulting recombinant viral particle in replication-permissive mammalian cells expressing the missing adenoviral replication genes to generate a recombinant replication-defective viral particle.

56. A method of generating a viral particle according to claim 55, wherein said identification step comprises the steps of:

- (i) monitoring transfected cells for evidence of cytopathic effect;
- (ii) isolating viral nucleic acid from the cell supernatant of cultures of the transfected cells showing a cytopathic effect;
- (iii) identifying successful recombinant viral vectors by PCR using primers complementary to the promoter operably linked to the beta-ASP gene and primers complementary to adenovirus sequences; and
- (iv) purifying the recombinant viral particles by plaque purification.

57. A recombinant pro-viral plasmid comprising a gene encoding a beta-ASP operably linked to a promoter and further comprising a replication-defective viral genome.

58. A recombinant pro-viral plasmid according to claim 57, wherein said beta-ASP is selected from the group consisting of a beta-AR, a GRK inhibitor and an adenylylcyclase.

59. A recombinant pro-viral plasmid according to claim 57, wherein said beta-ASP is adenylylcyclase isoform VI.

60. A recombinant pro-viral plasmid according to claim 57, wherein said beta-ASP is a chimeric adenylylcyclase.

61. A recombinant pro-viral plasmid according to claim 57, wherein said beta-ASP is the adenylylcyclase isoform VI of SEQ ID NO. 13.

62. A recombinant pro-viral plasmid according to claim 57, wherein said beta-ASP is adenylylcyclase human isoform VI.

63. A recombinant pro-viral plasmid according to claim 57, wherein said beta-ASP is the adenylylcyclase human isoform VI of SEQ ID 11.

64. A recombinant pro-viral plasmid according to claim 57, wherein the gene encoding a beta-ASP is a variant of a wild-type beta-ASP gene, the variant comprising a deletion in one or more untranslated regions of said beta-ASP gene.

5 65. A recombinant pro-viral plasmid according to claim 64, wherein said deletion removes at least about 100 bp of the 3'-untranslated region.

10 66. A recombinant pro-viral plasmid according to claim 64, wherein the gene encoding a beta-ASP is a variant AC gene having a deletion in the 3'-untranslated region.

67. A recombinant pro-viral plasmid according to claim 64, wherein the gene encoding a beta-ASP is a truncated AC_{VI} gene having a deletion removing the 3'-untranslated region.

15 68. A recombinant pro-viral plasmid according to claim 57, wherein said replication-defective viral genome is a replication-defective adenoviral genome.

69. A cell comprising a recombinant pro-viral plasmid according to claim 57.

70. A polynucleotide comprising a sequence encoding a chimeric adenylylcyclase polypeptide.

71. A polynucleotide of claim 70 encoding the AC_{VI} of SEQ ID NO. 13.

20 72. A polynucleotide of claim 70 comprising the nucleotide sequence of SEQ ID NO. 12.

73. An isolated polynucleotide comprising a sequence encoding a human adenylylcyclase VI (AC_{VI}) polypeptide, or a variant thereof having adenylylcyclase activity.

74. An isolated polynucleotide comprising a sequence encoding a human adenylylcyclase VI (AC_{VI}) polypeptide of SEQ ID NO. 11. *70*

25 75. An isolated polynucleotide comprising:
a sequence of at least 100 nucleotides that has at least 95% overall sequence identity with a nucleotide sequence of comparable length within the sequence shown SEQ ID NO. 1 or 3 or 5. *See pg 70*

30 76. An isolated polynucleotide of claim 75, wherein said overall sequence identity is at least 99%.

77. An isolated polynucleotide of claim 76, wherein said polynucleotide comprises a sequence of at least 1000 nucleotides having at least 95% overall sequence identity with a nucleotide sequence of comparable length within the sequence shown in SEQ ID NO. 1 or 3 or 5.

5 78. An isolated polynucleotide of claim 73, wherein said polynucleotide hybridizes at high stringency to a polynucleotide having the nucleotide sequence shown in SEQ ID NO. 1 or 3 or 5.

79. An isolated polypeptide encoded by the polynucleotide of claim 70.

80. An isolated polypeptide encoded by the polynucleotide of claim 71.

10 81. An isolated polypeptide encoded by the polynucleotide of claim 72.

82. An isolated polypeptide encoded by the polynucleotide of claim 73.

83. An isolated polypeptide encoded by the polynucleotide of claim 74.

84. An isolated polypeptide of claim 82, wherein said polypeptide comprises a sequence of at least 300 amino acid residues that has at least 95% overall amino acid sequence identity with a sequence of comparable length within the sequence shown in SEQ ID NO. 2 or 4 or 6.

15 85. An isolated polypeptide of claim 84, wherein said overall amino acid sequence identity is at least 99%.

86. A vector comprising a polynucleotide of claim 70.

87. A vector comprising a polynucleotide of claim 71.

20 88. A vector comprising a polynucleotide of claim 72.

89. A vector comprising a polynucleotide of claim 73.

90. A vector comprising a polynucleotide of claim 74.

25 91. A vector of claim 86, wherein said vector is selected from the group consisting of a viral vector and a lipid-based vector.

92. A vector of claim 86, wherein said vector is a replication-defective viral vector selected from the group consisting of an adenoviral vector and an adeno-associated viral vector.

30 93. A vector of claim 87, wherein said vector is selected from the group consisting of a viral vector and a lipid-based vector.

94. A vector of claim 87, wherein said vector is a replication-defective viral vector selected from the group consisting of an adenoviral vector and an adeno-associated viral vector.

5 95. A vector of claim 88, wherein said vector is selected from the group consisting of a viral vector and a lipid-based vector.

96. A vector of claim 88, wherein said vector is a replication-defective viral vector selected from the group consisting of an adenoviral vector and an adeno-associated viral vector.

10 97. A vector of claim 89, wherein said vector is selected from the group consisting of a viral vector and a lipid-based vector.

98. A vector of claim 89, wherein said vector is a replication-defective viral vector selected from the group consisting of an adenoviral vector and an adeno-associated viral vector.

99. A vector of claim 90, wherein said vector is selected from the group consisting of a viral vector and a lipid-based vector.

100. A vector of claim 90, wherein said vector is a replication-defective viral vector selected from the group consisting of an adenoviral vector and an adeno-associated viral vector.

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